Electronic supplementary materials *Mendeleev Commun.*, 2021, **31**, 512–514

Structure and cytotoxicity of biodegradable poly(D,L-lactide-*co*-glycolide) nanoparticles loaded with oxaliplatin

Ekaterina V. Razuvaeva, Kirill T. Kalinin, Nikita G. Sedush, Alexey A. Nazarov, Dmitry S. Volkov and Sergei N. Chvalun

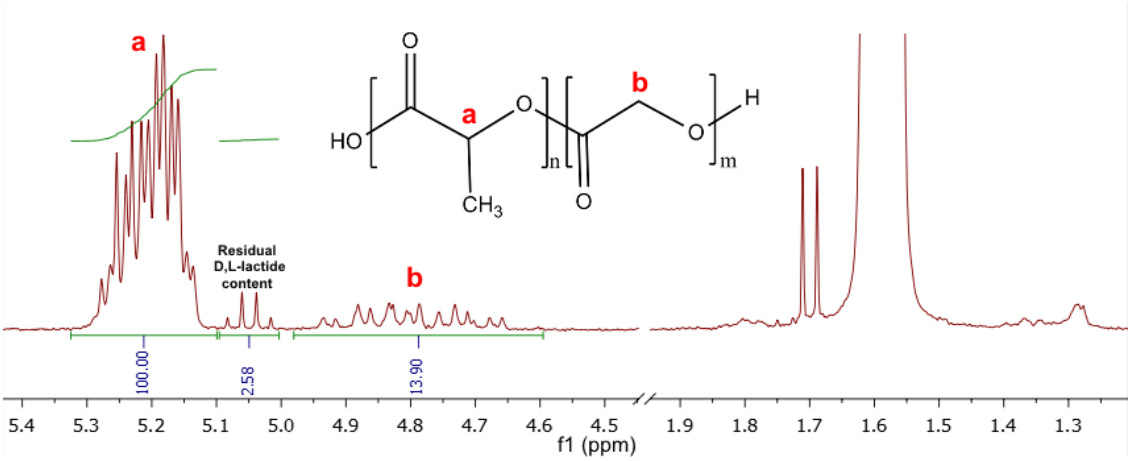


Figure S1 Fragment of ^{1}H NMR spectrum of the synthesized poly(D,L-lactide-*co*-glycolide) in deuterated chloroform.

Molar fractions of lactide (LA) and glycolide (GL) units in the copolymer were calculated from NMR spectrum (Figure S1) according to eq. 1 and 2:

$$(LA) = \frac{a}{a+0.5b} \times 100\% = 93.5\ \%, \quad (1)$$

$$(GL) = \frac{0.5b}{a+0.5b} \times 100\% = 6.5\ \%, \quad (2)$$

where *a* is integral intensity of –CH groups (δ = 5.10 – 5.30 ppm) and *b* is intensity of –CH_2 groups (δ = 4.60 – 4.97 ppm).

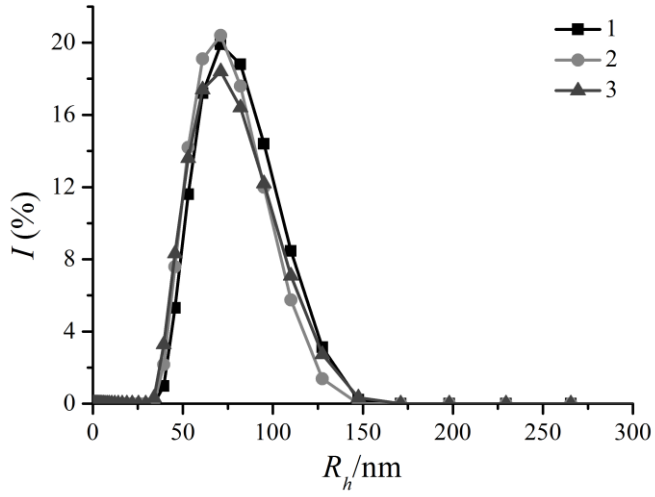
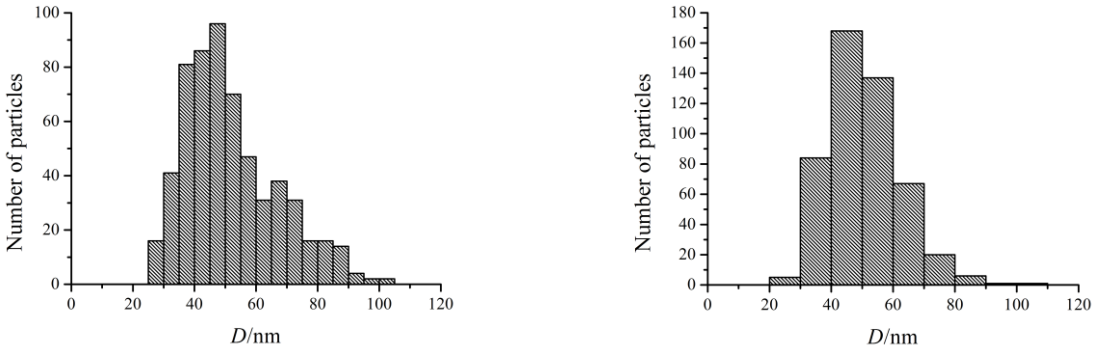


Figure S2 DLS intensity size distribution curves for (*1*) the drug-free PLGA nanoparticles and the oxaliplatin-loaded PLGA nanoparticles with drug loading content of (*2*) 0.26 and (*3*) 0.30 wt% (*c* = 0.1 g/L).



a **b**

Figure S3 Size distribution histograms of the PLGA nanoparticles evaluated from (**a**) AFM and (**b**) TEM images.

Materials and Methods

Materials. D,L-lactide and glycolide were purchased from Corbion (Netherlands) and recrystallized in butyl acetate before use. Stannous (II) 2-ethylhexanoate ($Sn(Oct)_2$), poly(vinyl alcohol) (PVA) with a weight-average molecular weight of 30-70 kDa (87% degree of hydrolysis), D-mannitol were purchased from Sigma-Aldrich (USA) and used as received. Oxaliplatin (trans-R,R-cyclohexane-1,2-diamine)oxalatoplatinum (II)) was synthesized using a procedure described

in literature.[1] All organic solvents were of analytical grade and used without further purification. Double distilled water was used for all experiments.

Synthesis of PLGA copolymer. Poly(D,L-lactide-*co*-glycolide) (90:10) was synthesized by ring-opening copolymerization of D,L-lactide with glycolide. Stannous (II) 2-ethylhexanoate (500 ppm) was used as a catalyst. The polymerization was carried out in melt for 2.5 h at a temperature of 180 °C. The reaction product was cooled to room temperature and dissolved in tetrahydrofuran then precipitated three times with *n*-hexane. The synthesized copolymer was dried under vacuum at low pressure for 24 h.

The composition of the synthesized PLGA copolymer was determined by ^{1}H nuclear magnetic resonance (^{1}H NMR) (Figure S1). The integrals of the peaks corresponding to the methylene protons of glycolide (-CH_2) and methine protons of D,L-lactide (-CH) were used to calculate the lactide:glycolide ratio (93:7 mol%). The content of residual monomer was determined as 2.6 mol%.

Gel permeation chromatography (GPC) was used to determine molecular weight and polydispersity index of the synthesized PLGA copolymer. The weight-average and number-average molecular masses of PLGA were evaluated as 168 and 70 kDa, correspondingly, whereas the polydispersity index was 2.4.

Preparation of PLGA nanoparticles. Aqueous suspensions of drug-free and drug-loaded PLGA nanoparticles were prepared by a nanoprecipitation technique. Briefly, PLGA (100 mg) was dissolved in acetone (10 ml). The obtained solution was added dropwise into the aqueous solution (10 ml) of PVA stabilizer with the concentration of 10 g/L. The organic solvent was removed throughout evaporation for 4 h at room temperature under stirring (700 rpm). Finally, the aqueous suspensions were centrifuged (40000 g, 30 min) to remove the residues of the organic solvent and free stabilizer. The precipitated nanoparticles were dispersed in water. The procedure was repeated three times.

To prepare oxaliplatin-loaded PLGA nanoparticles, 6 or 10 mg of oxaliplatin (3 or 5% wt/wt with respect to masses of copolymer and stabilizer) was preliminary dissolved in the aqueous phase.

Dynamic light scattering (DLS). The DLS measurements were performed with a Zetasizer Nano ZS instrument (Malvern Ltd.), equipped with a He-Ne laser with a wavelength of 633 nm at a scattering angle of 173°. Aqueous suspensions of the PLGA nanoparticles with concentration of 0.1 g/L were placed into a quartz cuvette with the optical length of 10 mm. The measurements were carried out at 25 °C. Analysis of the correlation functions was performed using the Zetasizer software.

Electrophoretic light scattering. The electrokinetic potential (ζ-potential) of the PLGA nanoparticles was determined by electrophoretic light scattering. The measurements were performed with a Zetasizer Nano ZS instrument (Malvern Ltd.). Aqueous suspensions of the PLGA nanoparticles with concentration of 0.1 g/L were placed into an U-shaped capillary cuvette. The measurements were carried out at 25 °C.

Atomic force microscopy (AFM). AFM imaging in air was performed using a Multimode 8 microscope with a Nanoscope V controller (Bruker) operating in the PeakForce Tapping QNM mode. The Bruker's sharp nitride levers (SNL-10) were used as AFM probes. The aqueous suspensions (20 μl) of the PLGA nanoparticles with concentration of 1 g/L were cast on freshly cleaved mica plates and dried at room temperature. Analysis of the AFM images was carried out using the ScopePhoto programme.

Transmission electron microscopy (TEM). TEM imaging was performed using a Titan 80-300 TEM/STEM (FEI) microscope at accelerating voltage of 300 kV with a BM-Ultrascan (Gatan) camera operating in the bright field mode. For TEM experiments, the negative staining procedure was used. Thin-carbon-film-coated copper TEM grids were glow-discharged for 10 s in the Pelco easiGlow system. A 3 μL droplet of the aqueous suspension with concentration of 1 g/L was

deposited to the carbon side of the grid and incubated for 1 min. Then, the carbon side of the grid was rinsed with 10 μL of distilled water, and right after that 10 μL of uranyl acetate solution with concentration of 0.5 wt% was applied to the grid and incubated for 30 s. After each step the excess of solution was removed by contacting the grid edge with filter paper. Then, the grid was dried for 30 min at ambient conditions.

Small-angle X-ray scattering (SAXS). Aqueous suspensions of the PLGA nanoparticles were studied by SAXS at the European Molecular Biology Laboratory (EMBL) on the storage ring PETRA III (DESY, Hamburg) on the EMBL-P12 beamline equipped with a 2D photon counting pixel X-ray detector Pilatus 2 M (Dectris). The scattering intensity, *I(s)*, was recorded in the range of the momentum transfer $0.02 < s < 4.5$ nm^{-1}, where $s = (4\pi \sin \theta)/\lambda$, 2θ is the scattering angle, and $\lambda = 0.124$ nm is the X-ray wavelength. The measurements were carried out at 23 °C using continuous flow operation over a total exposure time of 1 s divided into 20 × 50 ms individual frames to monitor for potential radiation damage (no radiation effects were detected). For each sample, 20 scattering curves were captured to improve the quality of obtained data. The data were corrected for the solvent scattering and processed using standard procedures with the program PRIMUS.[2] Data analysis was performed using the software suite ATSAS.[3] Pair distance distribution functions were calculated using the program GNOM.[4]

Evaluation of drug loading. The content of oxaliplatin (weight ratio of the drug to the copolymer and stabilizer) in the freeze-dried PLGA nanoparticles was determined by inductively coupled plasma atomic emission spectroscopy (ICP-AES). An axial ICP-AES 720-ES spectrometer (Agilent Technologies, USA) was used for measurements with a low flow axial quartz torch with 2.4 mm inner diameter injector tube (Glass Expansion, Australia), a double-pass glass cyclonic spray chamber (Agilent Technologies), a OneNeb nebulizer (Agilent Technologies, USA), and a Trident Internal Standard Kit (Glass Expansion). Samples were introduced manually to reduce washing volume, without preliminary digestion or dilution. The drug loading content

DLC and encapsulation efficacy EE of the oxaliplatin-loaded PLGA nanoparticles were calculated according to the following equations:

$$DLC = \frac{m_1^{OxPt}}{m_{NP}} \times 100\% \quad (1)$$

$$EE = \frac{m_1^{OxPt}}{m_0^{OxPt}} \times 100\%, \quad (2)$$

where m_1^{OxPt} is mass of incorporated oxaliplatin in nanoparticles, m_{NP} is mass of nanoparticles, and m_1^{OxPt} is initial mass of oxaliplatin.

Evaluation of cytotoxicity via MTT assay. The MCF7, A549, HCT116, SW480 and WI38 cell lines were cultured in Dulbecco modified Eagle's medium DMEM (Gibco, UK) with 10% fetal bovine serum (Gibco, USA) and antibiotics (PanEco, Russia) in 5% CO_2 at a temperature of 37 ºC. The oxaliplatin was pre-dissolved at 20 mM in dimethyl sulfoxide (DMSO) and added to the cell culture at the required concentration with maximum DMSO content of 0.5 v/v%. The nanoparticles were suspended in DMEM and added to the cell cultures. The cells in 96-well plates (7×10^3 cells/well) were treated with various concentrations of compounds and cisplatin at 37 ºC for 72 h. The cell viability was determined using the MTT assay as follows: the cells were incubated at 37º C for 50 min with 100 µl of 5 mg/ml solution of 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (Sigma-Aldrich, USA) in the cell culture medium. The supernatant was discarded, and formazan was dissolved in 100 µl of DMSO. The optical density of the solution was measured at 570 nm on a multiwell plate reader (Zenith 200 rt). The percentage of viable (i.e., MTT converting) cells was calculated from the absorbance of untreated cells (100%). Each experiment was repeated three times, each concentration was tested in three replicates.